REMARKS

The present invention relates to a method and a reagent for measuring alanine aminotranseerase activity.

In the Office Action of August 29, 2005, the Examiner objected to the abstract as containing more than one paragraph. Claims 1 - 7 and 10 were rejected under 35 U.S.C. § 112, first paragraph, with respect to the written description requirement, and particularly with respect to the lactate dehydrogenase activity inhibitor. Furthermore, claims 6 and 7 were rejected under 35 U.S.C. § 112, second paragraph, particularly with respect to the phrase "may contain".

Claims 1 - 8 and 10 were rejected under 35 U.S.C. § 102(b) based on U.S. Patent No. 4,241,179 (Madappally et al). Furthermore, claims 1 - 10 were rejected under 35 U.S.C. § 103(a) based on the Madappally et al reference.

In response, Applicant first notes that the Abstract has been amended to place the Abstract in the form of a single paragraph. Therefore, it is respectfully submitted that the objection has been obviated.

Applicant has also amended herein the specfication at page 6, lines 11 - 13, due to the discovery of a technical error due to a mistranslation of the Japanese specification of the present PCT application. It is respecfully submitted that entry of the amendment is proper for technical

accuracy. A copy of page 4 of the Japanese document, and page 6 of the present specification further showing the proper translation, are attached hereto.

Applicants have also amended the claims herein, by canceling original claims 1 - 10, and adding new claims 11 - 25, which new claims include further detailed recitations, based on which it is respectfully submitted that the claims should be considered in full compliance with all requirements of 35 U.S.C. § 112, and furthermore based on which it is respectfully submitted that the claimed invention neither anticipated nor obvious in view of the cited prior art.

Although the new claims 11 - 25 include further recitations, the general relationship betweeen the new claims and the previous claims is indicated in the table below.

New claims	Old claims
11	6 and 7
12	(newly added)
13 - 15	8 - 10
16	1
17	(newly added)
18 - 20	2 - 4
21 - 25	5

The claims of the present invention in accordance with this amendment, and the distinctions of the present invention *vis-a-vis* the cited prior art reference, are discusssed in further detail below.

The preamble of new independent claim 11, up to the phrase "the improvement comprising", is based on the disclosure in the specification, e.g., at page 1, lines 2 - 17 up from the bottom of the page.

The recitation "said substance being selected from the group consisting of oxamic acid, oxalic acid, oxalacetic acid, pyruvic acid, and phosphoenolpyruvic acid, and salts thereof" is is supported by the disclosure in the specification, e.g., at page 6, lines 9-13.

Support for claims 12 and 17 is found in the disclosure of the specification, e.g., at page 6, lines 17-23.

It is respectfully submitted that new claims 11 - 25 are in full compliance with 35 U.S.C. § 112, and are patentable over the Madappally et al reference for the reasons described in further detail below.

Rejection under 35 U.S.C. §102(b) or §103(a)

(1) Characteristic features of the present invention

Important features of the present invention reside in, but are not limited to, the provision of (i) an improvement invention of a known method for measuring an alanine aminotransferase (ALT) activity on the basis of the following reaction formulae (I) and (II):

$$\text{L-alanine} + \text{2-oxoglutaric acid} \xrightarrow{\text{ALT}} \text{L-glutamic acid} + \text{pyruvic acid} \quad \text{(I)}$$

$$\text{pyruvic acid} + \text{NADH} \xrightarrow{\text{LD}} \text{lactic acid} + \text{NAD} \quad \text{(II)}$$

and (ii) the use of "a substance having an activity of inhibiting a lactate dehydrogenase (LD) activity" (hereinafter referred to as LD inhibitor).

(2) Unexpected remarkable effects of the present invention obtained by the feature (ii)

As described on page 17, lines 17-22 of the present specification, due to the above feature (ii) of the present invention, an increased reagent blank reaction, i.e., an increased initial absorbance, can be suppressed [hereinafter referred to as effect (ii-1)], and thus accurate measured values of the ALT activity can be obtained [hereinafter referred to as effect (ii-2)] . Further, the reagent of the present invention exhibits an activity of stabilizing LD [hereinafter referred to as effect (ii-3)].

The effect (ii-1) may be seen by refernce to Figure 1 of the drawings. As shown in Figure 1, with the reagent containers open, the reagent blank activity of the reagent A (comparison)

increased with time, while the reagent blank activity of the reagent B (present invention) showed little change (see page 15, lines 13-16) of the specification.

The effect (ii-2) may be seen by reference to Figure 2 of the drawings. As shown in Figure 2, with respect to measured values of the pooled serum, the measured values of the ALT activity in the reagent A (comparison) increased with time, while the measured values of the reagent B (present invention) showed little change, with the reagent containers open (see page 16, lines 3-8) of the specification.

The effect (ii-3) may be seen by reference to Figure 3 of the drawings. As shown in Figure 3, the residual ratio of the LD activity of the reagent B (present invention) was higher than that in the reagent A (comparison) both at 37°C and at 25°C (see page 17, lines 12-15) of the specification.

In the present invention, LD is used as an essential component in the measuring system of the reaction formulae (I) and (II), and the reactions of the reaction formulae (I) and (II) are carried out in the presence of the LD inhibitor. The reason for the above surprising effects (ii-1) to (ii-3) obtained, in spite of the presence of the LD inhibitor, has not been finally determined, but a hypothesis is disclosed on page 11, line 1 to page 13, line 7 of the present specification.

(3) Madappally et al. reference (USP 4,241,179)

(3-1) Difference in reaction system

The Madappally et al. reference discloses a method for measuring the ALT activity. As disclosed in column 2, lines 1-4, the method disclosed in the Madappally et al reference is based on the following reaction formulae (I) and (III)

$$\text{L-alanine} + \text{2-oxoglutaric acid} \xrightarrow[(=\text{GPT})]{\text{ALT}} (=\alpha\text{-ketoglutarate}) + \underset{(=\text{L-glutamate})}{\text{L-glutamic acid}} + \underset{(=\text{pyruvate})}{\text{pyruvic acid}} \quad \text{(I)}$$

$$\underset{(=\text{L-glutamate})}{\text{L-glutamic acid}} + \text{NAD} + H_2O \xrightarrow{\text{GLDH}} \text{2-oxoglutaric acid} + \text{NAD} + NH_3 \quad \text{(III)}$$

In this connection, the terms in parentheses above are the terms used in the Madappally et al reference.

The present invention is different in the reaction system from the measuring method disclosed in the Madappally et al. reference.

(3-2) Use of LD inhibitor

The Madappally et al reference disclosed that "(a)lso present in biological fluids, particularly serum, are lactate and the enzyme lactate dehydrogenase (LDH), in variable concentration. Owing to the activity of the enzyme, these substances increase the content of

NADH in the assay medium. In the past, oxamate or oxalate has been added to the medium, to inhibit lactate dehydrogenase" (column 2, lines 47-53).

As is apparent from the above description, the object of adding the LD inhibitor to the assay medium in the Madappally et al reference is to avoid the effects of internal LD contained in biological fluids.

Considering the foregoing, the present invention is see to distinguish over the Madappally et al reference for several resons.

First, the use of the LD inhibitor in the measuring method on the basis of the reaction formulae (I) and (II) is neither disclosed nor suggested in the Madappally et al reference.

Second, Applicant submits that there is no motivation in the disclosure of the Madappally et al reference for one of ordinary skill in the art to decide to add the LD inhibitor to the measuring method on the basis of the reaction formulae (I) and (II), whereas LD is an essential component in the measuring method of the present invention.

Third, the unexpected advantageous remarkable effects (i-1), (i-2), and (ii-3) are not disclosed nor suggested in the Madappally et al reference.

Therefore, Applicant respectfully submits that the present invention using the LD inhibitor in the measuring method on the basis of the reaction formulae (I) and (II), and exhibiting such unexpected advantageous remarkable effects, would **not** be conceivable from the disclosure of the Madappally et al reference.

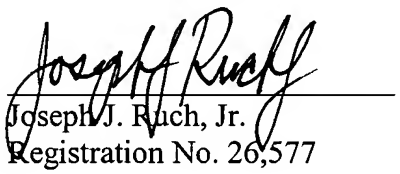
Accordingly, it is respectfully submitted that the Madappally et al reference should not be applied for rejection of new claims 11 - 25 under either of 35 U.S.C. § 102 or §103.

In view of the above, reconsideration and allowance of claims 11 - 25 of this application are now believed to be in order, and such actions are hereby earnestly solicited.

If any points remain in issue which the Examiner feels may be best resolved through a personal or telephone interview, the Examiner is kindly requested to contact the undersigned attorney at the telephone local Washington, D.C. number listed below.

The USPTO is directed and authorized to charge all required fees, except for the Issue Fee and the Publication Fee, to Deposit Account No. 19-4880. Please also credit any overpayments to said Deposit Account.

Respectfully submitted,



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Date: January 30, 2006

本発明の測定方法の好ましい態様においては、乳酸脱水素酵素活性に対する阻害作用を有する物質が、オキサミン酸又はその塩である。

本発明の測定方法の別の好ましい態様においては、オキサミン酸又はその塩の濃度が、測定系における終濃度として、０．００５～５ｍｍｏｌ／Ｌである。

本発明の測定方法の更に別の好ましい態様においては、乳酸脱水素酵素の濃度が、測定系における終濃度として、１００Ｕ／Ｌ以上である。

図面の簡単な説明

図１は、本発明及び比較用のＡＬＴ活性測定試薬における、ブランク感度の経時的変化を示すグラフである。

図２は、本発明及び比較用のＡＬＴ活性測定試薬を用いて測定した、プール血清中ＡＬＴ活性測定値の経時的変化を示すグラフである。

図３は、本発明及び比較用のＡＬＴ活性測定試薬における、ＬＤ安定性を示すグラフである。 oxamic acid①, oxalic acid②, oxalacetic acid③, pyruvic acid④, phosphoenolpyruvic acid⑤,

発明を実施するための最良の形態

本発明のＡＬＴ活性測定試薬は、Ｌ－アラニン、２－オキソグルタル酸、ＬＤ、及びＮＡＤＨを含む公知のＡＬＴ活性測定試薬の改良試薬である。Ｌ－アラニン、２－オキソグルタル酸、ＬＤ、及びＮＡＤＨを含むＡＬＴ活性測定試薬では、Ｌ－アラニン及び２－オキソグルタル酸を基質として、ＡＬＴによって生成されるピルビン酸をＬＤによって乳酸に変え、共存させておいたＮＡＤＨ量の減少量を、あるいは、生成するＮＡＤの増加量を、波長３４０ｎｍ付近で測定することにより、ＡＬＴ活性を測定することができる。

本発明のＡＬＴ活性測定試薬は、これらの公知の構成成分に加え、ＬＤ活性に対する阻害作用を有する物質（以下、ＬＤ阻害剤と称する）を含む。本発明で用いるＬＤ阻害剤としては、特に限定されるものではないが、例えば、オキサミン酸①、シュウ酸②、オキサロ酢酸③、ピルビン酸④、ホスホエノールピルビン酸⑤、ドデシル硫酸ナトリウム⑥、乳酸⑦、若しくはヒドロキシグルタル酸⑧、又はそれらの塩を用いることができ、ＡＬＴ活性測定に誤差を与えることがない点で、オキサミン酸

sodium dodecyl sulfate⑥, lactic acid⑦, or hydroxyglutaric acid⑧,

sodium dodecyl sulfate, lactic acid, or hydroxyglutaric acid,

generated from L-alanine and 2-oxoglutaric acid as substrates by ALT, is changed to lactic acid by LD, and a decreased amount of NADH coexisting or an increased amount of NAD generated is measured at the wavelength of approximately 340 nm.

The reagent for measuring an ALT activity of the present invention comprises a substance having an activity of inhibiting an LD activity (hereinafter referred to as an LD inhibitor) in addition to the known components. The LD inhibitor used in the present invention is not particularly limited, but there may be mentioned, for example, oxamic acid, oxalic acid, oxalacetic acid, pyruvic acid, or → Delete phosphoenolpyruvic acid, or salts thereof. Oxamic acid or a salt thereof, such as sodium salt, potassium salt, or lithium salt, usually does not lead to errors in measuring an ALT activity, and thus is preferable.

A concentration of the LD inhibitor contained in the reagent for measuring an ALT activity of the present invention may be changed in accordance with the kind of the LD inhibitor used, and thus is not particularly limited, so long as it is the concentration exhibiting the LD activity which does not affect a reagent for measuring an ALT activity. The final concentration in a measuring system may be generally 0.001 to 100 mmol/L, obtained by adjusting the concentration contained in the measuring reagent.

The term "exhibiting the LD activity which does not affect a reagent for measuring an ALT activity" as used herein means that at least an LD activity capable of measuring the ALT activity remains in a sample to be analyzed. Even if the LD activity is inhibited by the LD inhibitor, such a reagent for measuring an ALT activity can be used, so long as a minimum LD activity enough for measurement remains. In addition, the object of the present invention is to suppress the reagent blank reaction, and